## Appendix J

**Paper #19, 9/15/99 amendment, and paper #12, 12/22/98 amendment in Krieg '646 File History**

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ATTORNEY'S DOCKET NO. C1039/7004 (HCL)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicant: | Krieg, et al. |
| Serial No: | 08/738,652 |
| Filed: | October 30, 1996 |
| For: | IMMUNOSTIMULATORY NUCLEIC ACID MOLECULES |
| Art Unit: | 1633 |
| Examiner: | J. Martinell |

BOX CPA
ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

Sir:

**PRELIMINARY AMENDMENT**

Before calculating the fees, please amend the above-identified application as follows:

**In the claims**:

Please cancel claims 15, 16, and 21-24.

Please amend the claims as follows:

20. (Amended) A composition comprising:

a plasmid including an immunostimulatory nucleic acid sequence, having the following formula:

$$5'X_1X_2CGX_{[2]3}X_43'$$

[wherein the immunostimulatory nucleic acid includes at least 8 nucleotides and] wherein C and G are unmethylated, wherein $X_1$, [and] $X_2$, $X_3$ and $X_4$ are nucleotides and [wherein at least one nucleotide has a phosphate backbone modification, and]

an antigen in a pharmaceutically acceptable carrier.

18. (Amended) The composition of claim 20, [wherein the immunostimulatory nucleic acid has the following formula:

5′ $X_1X_2CGX_3X_4$ 3′]

wherein $X_1X_2$ are nucleotides selected from the group consisting of: GpT, GpG, GpA and ApA; and $X_3X_4$ are nucleotides selected from the group consisting of: TpT, CpT [or], GpT, and TpG.

26. (Amended) The composition of claim 20, further comprising a B-cell targeting molecule [that promotes high affinity binding to a target B cell].

28. (Amended) The composition of claim 20, wherein the antigen is encoded by a nucleic acid sequence in a plasmid [a nucleic acid encoding an antigen].

38. (Amended) The composition of claim 36, wherein the antigen is encoded by a nucleic acid sequence in a plasmid [a nucleic acid encoding an antigen].

42. (Amended) The composition of claim 40, wherein the antigen is encoded by a nucleic acid sequence in a plasmid [a nucleic acid encoding an antigen].

**In the Specification**

Please amend the specification as follows:

On page 1, the paragraph beginning at line 5, please delete the phrase "both of which are incorporated herein by reference in their entirety" added by amendment on December 22, 1998.

On page 19, line 10, please delete "GGGGTCAACGTTCAGGGGGG" and replace it with --GGGGTCAACGTTGAGGGGGG--.

On page 34, line 15, please delete "GGGGTCAACGTTGACGGGGG" and replace it with --GGGGTCAACGTTGAGGGGGG--.

On page 57, line 1, please delete "GGGGTCAACGTTCAGGGGGG" and replace it with --GGGGTCAACGTTGAGGGGGG--.

## REMARKS

These remarks are presented in response to the Office Action dated March 15, 1999 and also in response to the telephone conference with Examiner James Martinell on April 29, 1999. Claims 15, 16, and 21-24 have been canceled. Claims 20, 25, 26, 28, 38, and 42 have been amended. Support for the claim amendments can be found throughout the specification. No new matter is added by these amendments. The specific support for each amendment is discussed in more detail below.

### Telephone Interview With Examiner James Martinell

Initially, applicants express thanks to Examiner Martinell for his courtesy in granting and conducting a telephone interview with applicants' representative on April 29, 1999. The issue of the rejection of claims 12 and 14-47 under 35 U.S.C. §112 and of claims 15 and 16 under 35 U.S.C. §102 were discussed in the interview with the Examiner. In summary, it was agreed that applicants would cancel claim 15, as this claim is being pursued in a divisional application. Several amendments to the claim were also discussed in the interview with the Examiner in order to overcome the Section 112 rejections. The Examiner indicated that he was favorably impressed with the proposed claim amendments submitted prior to the interview and with the recitation of support in the specification in response to the remaining rejections discussed during the interview. Applicants have now formally amended the claims as discussed during the interview. Some additional amendments have been made, resulting from the allowance of related co-pending patent application Serial No. 08/386,063. (A copy of the claims as allowed is attached hereto as Exhibit 4, for the Examiner's reference.) This issue is discussed in more detail below.

Additionally, during the telephone interview, applicants' representative had indicated that a response after final would be filed. Following the interview, some sequence errors were discovered by the inventors in the specification. In order to correct the errors, it was necessary that a Declaration be filed. As a result, applicants representative contacted Examiner Martinell to inform him that applicants would file a continued prosecution application in order to submit the



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Declaration correcting the sequence errors.

**Declaration of Dr. Arthur Krieg**

Enclosed herewith is a Declaration of Dr. Arthur Krieg under 37 CFR §1.132. In the Declaration, Dr. Arthur Krieg indicates that an error in the sequence of SEQ ID NO:12 was discovered in the above-identified patent application. The error was a typographical error generated during the preparation of the patent application in which a G was substituted with a C. Dr. Krieg has maintained all of his records including his original order form requesting the preparation of an oligonucleotide, the shipping statement indicating that the oligonucleotide was received and a log book in which the correct sequence for the oligonucleotide was entered. All of the experiments described in the above-identified patent application which were performed using the oligonucleotide of SEQ ID NO:12 were performed with an oligonucleotide having the sequence GGGGTCAACGTTGAGGGGGG. The documentation submitted with the Declaration supports applicants' assertion that the oligonucleotide sequence described in the specification was incorrect. These documents also support the notion that the applicant had possession of the correct sequence at the time the application was filed. It is believed that this is sufficient supporting evidence to amend the sequence in the specification. Upon acceptance of the Declaration by the Examiner, applicants will submit a new sequence listing and disk.

**Amendment of Claim 20**

Claim 20 has been amended because of the allowance of similar claims in U.S. Patent Application Serial No. 08/386,063. Claim 20 has been amended to recite a composition of a plasmid which includes an immunostimulatory nucleic acid sequence having the formula 5' $X_1X_2CGX_3X_4$3' and an antigen in a pharmaceutically acceptable carrier. Support for the amendment can be found throughout the specification but, in particular, on page 14, lines 32-36, which teach that the immunostimulatory CpG DNA sequence can be present in a plasmid and on page 17, lines 12-22, which describes plasmids and vectors. Support for the formula 5'$X_1X_2CGX_3X_4$3' is found at least on page 14, lines 29-31 and in original claim 25. Claims 21-24



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were canceled because the limitations of these dependent claims were not valid with respect to a plasmid.

**Rejection of Claims 23, 24, 26-28, 34, 36-39, 42, and 44-47 Under 35 USC §112, Second Paragraph**

Claims 23 and 25 have been rejected under 35 USC §112, Second Paragraph as being vague and indefinite because of the recitation "wherein the phosphate backbone modification occurs at the 5'(3') end of the nucleic acid". Both claims 23 and 24 have been canceled and it is respectfully requested that this rejection be withdrawn.

Claim 26 has been rejected because the recitation of "high affinity binding" is vague and indefinite. Applicants have amended claim 26 as presented in the proposed amendments discussed during the telephone interview. It is believed that the amendment is sufficient to overcome the rejection.

Claims 28, 34, 38, and 42 have been rejected as being vague, indefinite, misdescriptive, and inaccurate in reciting the phrase "wherein the antigen is a nucleic acid encoding an antigen" because "the claim implies that the antigen is a nucleic acid that is not the antigen." Although applicants had previously planned to cancel these claims for other reasons, it has been decided to maintain these claims. Applicants have amended the claim to correct the Section 112 problem. The claim now recites that the antigen is encoded by a nucleic acid sequence in a plasmid vector. It is believes that this corrects the Section 112 problem and that the claim no longer implies that the antigen is a nucleic acid. It is respectfully requested that this rejection be withdrawn.

Claim 36 has been rejected because of the recitation of "synthetic" in the claim. According to the Examiner "the instant application does not distinguish a synthetic nucleic acid from a non-synthetic nucleic acid". It was discussed in the telephone conference that the term "synthetic" could be canceled from the claim to broaden the scope of the claims. Applicants had planned to cancel the word "synthetic" but in view of the claims allowed in parent patent application Serial No. 08/386,063, this amendment is no longer appropriate. The support for the term "synthetic" in the specification was also discussed in the telephone interview. Because



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applicants are no longer canceling the term "synthetic" from the claim, applicants hereby set forth a description of the support within the specification for this term. On page 15, lines 30-34 of the specification, it is stated that the "nucleic acid molecules can be obtained from existing nucleic acid sources (e.g.. genomic or cDNA), but *are preferably synthetic* (e.g., produced by oligonucleotide synthesis)." (Emphasis added). Additionally, procedures for synthesizing and isolating nucleic acids are described on page 39. Applicants believe that this support is sufficient to overcome the rejection of the phrase "synthetic".

**Rejection of Claims 12 and 14-47 Under 35 USC §112, First Paragraph**

Claims 12 and 14-47 have been rejected under 35 USC §112, first paragraph, as containing subject matter which was not described in the specification in such a way to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention". The rejection is based on the phrase "wherein $X_1$ and $X_2$ are nucleotides" and because applicants have not pointed to the basis in the application for the change. This rejection was discussed in the telephone interview with the Examiner and it was agreed that support for this limitation could be found on page 7, lines 20-31 as well as page 14, lines 16-31. These sections of the specification describe preferred embodiments in which the immunostimulatory nucleic acid contains a consensus CpG motif and a formula of 5'$X_1CGX_2$3' or 5'$X_1X_2CGX_3X_4$3'. In line 31 of page 7 and 14 $X_1$, $X_2$, $X_3$, and $X_4$ are defined as being nucleotides. It is believed that this support, as discussed in the telephone interview, is sufficient to overcome the rejection.

**Rejection of Claims 15 and 16 Under 35 USC §102**

Claims 15 and 16 have been rejected under 35 USC §102 as being anticipated by Tokunaga et al. It is applicants' belief that the Tokunaga et al. reference does not anticipate either claim 15 or 16 because Tokunaga does not disclose that an immunostimulatory oligonucleotide can redirect a subject's immune response from a $Th_2$ to a $Th_1$ response or that these oligonucleotides would be useful for treating asthma. Tokunaga only discloses

oligonucleotides which augment NK cell activity and induce MAF and IFN. Redirection of an immune response or treatment of an asthma are not disclosed. Although applicants believe that claims 15 and 16 as written are patentable in view of the prior art, these claims are being canceled herewith and are being pursued in divisional applications. Claim 15 is now being pursued in divisional U.S. Patent Application Serial No. 09/337,893 and claim 16 is now being pursued in divisional U.S. Patent Application Serial No. 09/337,584.

## SUMMARY

It is believed that the claims are now in condition for allowance. A favorable action is earnestly solicited. If for any reason the Examiner has any questions or requires further information, he is encouraged to contact the applicants' representative at the number presented below.

Respectfully Submitted,

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Attorney Docket No: C1039/7004
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**x9/15/99**

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ATTORNEY DOCKET C1039/7004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicant : | Krieg et al. | Art Unit: 1633 |
| Serial No.: | 08/738,652 | Examiner: J. Martinell |
| Filed : | 10/30/96 | |
| Title : | IMMUNOSTIMULATORY NUCLEIC ACID MOLECULES | |

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Assistant Commissioner for Patents, Washington, D.C. 20231, on the 22nd day of December, 1998.

Helen C. Lockhart

Helen C. Lockhart, Reg. No. 39,248

Assistant Commissioner of Patents
Washington, DC 20231

**AMENDMENT**

In response to the Office Action dated, June 23, 1998, in connection with the above-identified patent application, the time for responding having been extended to December 23, 1998, by the accompanying fee, please consider the following amendments and remarks:

**In the Claims:**

Please amend the claims as follows:

12. (Amended) A method for ameliorating an immune system deficiency in a subject, comprising the steps of:

a) contacting lymphocytes obtained from the subject with an antigen and an [composition of claim 1] immunostimulatory nucleic acid, having the following formula:

5' $X_1CGX_2$ 3'



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wherein the immunostimulatory nucleic acid includes at least 8 nucleotides and wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides and wherein the immunostimulatory nucleic acid is an immunostimulatory nucleic acid selected from the group consisting of a synthetic immunostimulatory nucleic acid and an immunostimulatory nucleic acid having a phosphate modified backbone *ex vivo*, thereby producing activated lymphocytes; and

b) readministering the activated lymphocytes obtained in step a) to the subject.

14. (Amended) A method of claim 12 [or 13], wherein the immune system deficiency is selected from the group consisting of: the presence of a tumor, cancer or infectious agent in the subject.

15. (Amended) A method for redirecting a subject's immune response from a Th2 to a Th1 comprising the step of administering to the subject an [nucleic acid of claim 1] immunostimulatory nucleic acid, having the following formula:

5' $X_1CGX_2$ 3'

wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides, wherein the nucleic acid has between only 8 and 100 nucleotides.

16. (Amended) A method for ameliorating asthmatic symptoms in a subject, comprising administering to the subject [a nucleic acid of claim 1] an immunostimulatory nucleic acid, having the following formula:

5' $X_1CGX_2$ 3'

wherein the immunostimulatory nucleic acid includes at least 8 nucleotides and wherein C and G are unmethylated and wherein $X_1$ and $X_2$ are nucleotides.

17. (Amended) A method for desensitizing a subject against the occurrence of an allergic reaction in response to contact with a particular allergen, comprising administering to the subject an

effective amount of [the nucleic acid of claim 1] an immunostimulatory nucleic acid, having the following formula:

5′ $X_1CGX_2$ 3′

wherein the immunostimulatory nucleic acid includes at least 8 nucleotides and wherein C and G are unmethylated and wherein $X_1$ and $X_2$ are nucleotides and an effective amount of the allergen.

18. (Amended) A[n improved] method of vaccination in a subject, comprising administering to the subject a vaccine antigen or a nucleic acid encoding the vaccine antigen and [a nucleic acid of claim 1]an immunostimulatory nucleic acid, having the following formula:

5′ $X_1CGX_2$ 3′

wherein the immunostimulatory nucleic acid includes at least 8 nucleotides and wherein C and G are unmethylated and wherein $X_1$ and $X_2$ are nucleotides.

19. (Amended) A[n improved] method for treating leukemia in a subject, comprising administering to the subject [a nucleic acid of claim 1]an immunostimulatory nucleic acid, having the following formula:

5′ $X_1CGX_2$ 3′

wherein the immunostimulatory nucleic acid includes at least 8 nucleotides and wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides, prior to or in conjunction with a chemotherapy, so that the subject's leukemia cells are more sensitive to the chemotherapy.

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Please cancel claims 1-11 and 13 without prejudice and add the following new claims:

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20. A composition comprising:

an immunostimulatory nucleic acid, having the following formula:

5′ $X_1CGX_2$ 3′

wherein the immunostimulatory nucleic acid includes at least 8 nucleotides and wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides and wherein at least one nucleotide has a phosphate backbone modification, and

an antigen in a pharmaceutically acceptable carrier.

21. The composition of claim 20, wherein the immunostimulatory nucleic acid molecule is 8 to 100 nucleotides in length.

22. The composition of claim 20, wherein the phosphate backbone modification is a phosphorothioate or phosphorodithioate modification.

23. The composition of claim 20, wherein the phosphate backbone modification occurs at the 5′ end of the nucleic acid.

24. The composition of claim 20, wherein the phosphate backbone modification occurs at the 3′ end of the nucleic acid.

25. The composition of claim 20, wherein the immunostimulatory nucleic acid has the following formula:

5′ $X_1X_2CGX_3X_4$ 3′

wherein $X_1X_2$ are nucleotides selected from the group consisting of: GpT, GpG, GpA and ApA; and $X_3X_4$ are nucleotides selected from the group consisting of: TpT, CpT or GpT.

26. The composition of claim 20, further comprising a targeting molecule that promotes high affinity binding to a target B cell.

~~27.~~ The composition of claim ~~26~~, wherein the targeting molecule is selected from the group consisting of a sterol, a lipid and a B-cell specific binding agent.

28. The composition of claim 20, wherein the antigen is a nucleic acid encoding an antigen.

~~29.~~ The composition of claim ~~20~~, wherein the antigen is selected from the group consisting of proteins, polysaccharides, polysaccharide conjugates, glycolipids, viruses, bacteria, fungi, parasites, and allergens.

~~30.~~ The composition of claim ~~29~~, wherein the antigen is an allergen.

~~31.~~ The composition of claim ~~29~~, wherein the antigen is derived from an infectious organism selected from the group consisting of infectious bacteria, infectious virus, and infectious fungi.

32. A composition comprising:

an isolated immunostimulatory nucleic acid of 8 to 100 nucleotides in length, having the following formula:

$$5' X_1CGX_2 3'$$

wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides, and

an antigen in a pharmaceutically acceptable carrier.

~~33.~~ The composition of claim ~~32~~, wherein the isolated immunostimulatory nucleic acid includes a phosphate backbone modification which is a phosphorothioate or phosphorodithioate modification.

34. The composition of claim 32, wherein the antigen is a nucleic acid encoding an antigen.

35. The composition of claim 32, wherein the antigen is selected from the group consisting of proteins, polysaccharides, polysaccharide conjugates, glycolipids, viruses, bacteria, fungi, parasites, and allergens.

36. A composition comprising:

a synthetic immunostimulatory nucleic acid having the following formula:

5′ $X_1CGX_2$ 3′

wherein the immunostimulatory nucleic acid includes at least 8 nucleotides and wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides, and

an antigen in a pharmaceutically acceptable carrier.

37. The composition of claim 36, wherein the synthetic immunostimulatory nucleic acid includes a phosphate backbone modification which is a phosphorothioate or phosphorodithioate modification.

38. The composition of claim 36, wherein the antigen is a nucleic acid encoding an antigen.

39. The composition of claim 36, wherein the antigen is selected from the group consisting of proteins, polysaccharides, polysaccharide conjugates, glycolipids, viruses, bacteria, fungi, parasites, and allergens.

40. A composition comprising:

an immunostimulatory nucleic acid of 8 to 40 nucleotides in length, having the following formula:

$$5' X_1CGX_2 3'$$

wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides, and

an antigen in a pharmaceutically acceptable carrier.

41. The composition of claim 40, wherein the immunostimulatory nucleic acid includes a phosphate backbone modification which is a phosphorothioate or phosphorodithioate modification.

42. The composition of claim 40, wherein the antigen is a nucleic acid encoding an antigen.

43. The composition of claim 40, wherein the antigen is selected from the group consisting of proteins, polysaccharides, polysaccharide conjugates, glycolipids, viruses, bacteria, fungi, parasites, and allergens.

44. A method of inducing an antigen-specific immune response in a subject comprising:

administering a vaccine to a subject, wherein the vaccine includes an antigen in combination with an immunostimulatory nucleic acid of claims 26, 32, 36 or 40 in an amount effective to induce an immune response.

45. The method of claim 44, wherein the antigen is selected from the group consisting of proteins, polysaccharides or polysaccharide conjugates, glycolipids, viruses, bacteria, fungi, parasites, and allergens.

46. The method of claim 44, wherein the vaccine is administered *ex vivo*.

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~~47~~. The method of claim ~~45~~, wherein leukocytes of the subject are isolated and contacted with the antigen and immunostimulatory nucleic acid to produce activated leukocytes and wherein the activated leukocytes are readministered to the subject.

**In the Specification**

Please amend the specification as follows:

On page 12 line 11, please replace "genuses" with --genera--.

On page 1, line 5, please insert --This application is a continuation of co-pending U.S. Patent Application serial number 08/386,063, filed 2/7/95 now currently pending, which is a continuation-in-part of co-pending U.S. Patent Application 08/276,358, filed 7/15/94 which is abandoned, ~~both of which are incorporated herein by reference in their entirety~~.--

## REMARKS

These remarks are in response to the Office Action dated June 23, 1998. Please cancel claims 1-11 and 13. Please amend claims 12-19 and add new claims 20-47. Support for the claim amendments and for new claims 20-47 can be found throughout the specification, particularly on page 40, lines 19-34, and page 51, line 1 to page 52, line 14. No new matter is added by these amendments. Applicant's amended claims more clearly reflect the inventive discovery that a CpG-containing oligonucleotide in combination with an antigen, regardless of the choice of antigen, is useful for inducing antigen-*specific* immune response. Applicant has discovered a method for inducing an antigen-specific immune response. Applicant respectfully requests reconsideration of the present application.

## REJECTIONS UNDER 35 U.S.C.§112

Claims 1-19 were rejected under 35 U.S.C.§112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The pending claims have been amended as suggested by the Examiner.

REJECTIONS UNDER 35 U.S.C.§102 and 103

Claims 1-3, 4-7, 9-11, 13, and 15 were rejected under 102(a) or (b) as being anticipated by (a) Promega Catalog (1993/1994), p. 90, (b) New England Biolabs Catalog, p. 87-89, and 95 (1993/1994), (c) Tullis, U.S. Patent No. 5,023,243, (d) Sato et al., Science 273: 352 (1996), (e) Hutcherson et al., U. S. Patent No. 5,663,153, (f) Hoke et al., U.S. Patent No. 5,585,479, (g) Draper et al., U.S. Patent No. 5,248,670, (h) Hutcherson et al. WO 95/2604, (i) Androphy et al. EP 0 302 758, (j) Branda et al., Biochem. Pharmacol. 45: 2037 (1993), (k) Tokunaga et al., Jpn. J. Cancer Res. (Gann.) 79: 682 (1988), (l) Yamamoto et al., Antisense Res. Dev. 4: 199 (1994), (m) Yamamoto et al., Microbiol. Immunol. 38: 831 (1994), (n) Yamamoto et al., Jpn. J. Cancer Res. 85: 775 (1994), (o) Yamamoto et al., J. Immunol. 148: 4072 (1992), or, (p) New England Biolabs Catalog (1988/1989) as each of these publications allegedly discloses oligonucleotides having the sequence GC (sic) and/or the use of these oligonucleotides for immunostimulation.

Claims 1-11 and 13 have been canceled and claims 12 and 14-19 have been amended. Amended claims 12 and 14-19 and new claims 20-47 distinguish over the prior art of record. Applicants present below a discussion of each of the pending claims with respect to the prior art of record.

New claim 20 recites a pharmaceutical composition of an immunostimulatory nucleic acid, having the formula 5' $X_1CGX_2$ 3' wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides and wherein at least one nucleotide has a phosphate backbone modification. Claim 20 also recites the limitation that the pharmaceutical composition includes an antigen. Claims 21-31 depend from claim 20.

None of the references listed above discloses an oligonucleotide having a phosphate backbone modification specifically in combination with an antigen of interest, and thus none of the references anticipate the claims.

The new claims are not obvious in view of the cited prior art because the combination of references does not provide a disclosure which would teach one of ordinary skill in the art that oligonucleotides having the formula 5' $X_1CGX_2$ 3' wherein C and G are unmethylated and wherein

$X_1$ and $X_2$ are nucleotides are useful in combination with an antigen for producing an antigen-specific immune response. None of the references disclose a pharmaceutical composition including the claimed oligonucleotide and an antigen.

Most of the references described above were cited to demonstrate that unmethylated CG oligonucleotides have been described in the prior art before Applicants' priority date. Applicants do not dispute this fact. The prior art, however, does not show or suggest that the claimed oligonucleotides could be used together with any antigen to yield an enhanced specific immune response to the antigen. It simply was not predictable that the claimed oligonucleotides could be used as adjuvants with antigens to generate specific and strong immune responses to the antigen. The cited art does not relate to generating a specific immune response. It relates, instead, to the field of antisense, to the generation of nonspecific immune responses, which are inapplicable to and nonpredictive of the present invention as claimed.

Several of the prior art references describe specific palindromic oligonucleotides that produce a non-specific immune response, such as the induction of natural killer cells or interferon expression. These references are not predictive of and do not render obvious the findings of the instant invention that CpG containing oligonucleotides can induce an antigen-specific immune response. In particular, the following references fall into this category of prior art:

Tokunaga et al., Jpn. J. Cancer Res. (Gann.) 79: 682 (1988), reports that poly(dG, dC) induces interferon production and natural killer activity. According to the reference it was previously shown that a fraction designated MY-1 from BCG which was composed of 70% DNA, 28% RNA, 1.3% protein, 0.27% hexose, and 0.1% lipid augmented natural killer activity and produced interferon and demonstrated a strong in vivo anti-tumor activity. These physiological effects were postulated to be due to the DNA since digestion of the fraction with DNase lost its activity. To determine whether this activity was due to DNA, synthetic DNA polymers were prepared and tested in this paper. Table 1 shows all of the different types of synthetic oligonucleotides that were synthesized and tested for natural killer, macrophage activating factor (MAF), and interferon activity. Four different oligonucleotide types were found to augment natural



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killer activity and induce MAF and interferon similar to RNA, poly(rI)poly(rC). Poly(dG, dC) and RNA polymers were found to augment natural killer activity and induce MAF and interferon.

Yamamoto et al., J. Immunol. 148: 4072 (1992), describes palindromes in oligonucleotides which induce interferon and NK activity. The palindromic sequences were used in various tests to identify the mechanisms leading to this immune stimulation. By producing smaller nucleotides containing the palindromic sequence, 15-mer oligonucleotides were found to not have any activity, suggesting that a molecular size of approximately 30 bases is required for the oligonucleotides to induce biological activity. All of the active oligonucleotides contained one or more palindromic sequences whereas the inactive ones did not. It was found that the activity of oligonucleotides with GACGTC palindromic sequence were stronger than those without the sequence. It was also found that introduction of CACGTC, AGCGCT, AACGTT, or GACGTC but not ACCGGT into inactive oligonucleotides turn them into active ones. The oligonucleotides also lost their activity when a nucleotide within the palindrome was substituted and did not when one outside the palindrome was substituted. The authors indicated that of the 64 possible hexameric palindromes only 9 oligonucleotides supported the immunostimulatory activity of the 30-mer nucleotide. Table VI shows the effect of changing mononucleotides on the natural killer cell stimulatory activity of the oligonucleotides.

Yamamoto et al., Jpn. J. Cancer Res. 85: 775 (1994) discloses that oligonucleotides having palindromic sequences AACGTT and GACGTC increase interferon production in human peripheral blood lymphocytes. The specific oligonucleotides delivered in the liposomes are shown in Table 1.

Yamamoto et al., Microbiol. Immunol. 38: 831 (1994) show that oligonucleotides having the sequence AACGTT, when delivered in liposomes to murine splenocytes, increase interferon production and produce natural killer activity. Oligonucleotides having the sequence ACCGGT are inactive in this assay. The specific sequences used are shown on page 831.

Yamamoto et al., Antisense Res. Dev. 4: 199 (1994) shows that the sequence AACGTT, when administered in liposomes to murine splenocytes, increase interferon production. The specific oligonucleotides tested are shown in Fig. 1.

The Yamamoto references do not render claim 20 and the claims dependent thereon obvious because these references do not disclose or suggest that the oligonucleotides can be administered in combination with antigen. All of the assays demonstrate only general stimulation of natural killer cell activity resulting from the increased production of interferon. Antigen stimulation to produce an antigen-specific response is not a component of any of these assays: antigen is not utilized nor is the production of an antigen-specific response demonstrated or likely to result. The specification of the instant invention describes the use of oligonucleotides of the invention in combination with antigen and the production of an antigen-specific response on page 40, lines 19-34. Therefore, claim 20 would not have been obvious at the time of the invention.

Another class of prior art references which describe immune activation in response to oligonucleotides are papers describing antisense effects. In general these publications describe the observation that some antisense oligonucleotides produce an unwanted side effect of immune stimulation. Several of these publications conclude that these immune response side effects of the oligonucleotides should be avoided when performing antisense methods. Others describe the immune stimulatory effect as resulting from the antisense effects of the oligonucleotides used. These references do not, however, describe the combination of antigen and oligonucleotide for stimulating an antigen-specific immune response.

The Branda et al. reference (Biochem. Pharmacol. 45: 2037 (1993)) falls within this category of references. Branda describes specific antisense oligonucleotides to the HIV *rev* gene that causes immune stimulation in mice with B cell proliferation and Ig secretion. Branda performs studies and concludes that an antisense oligonucleotide is a mitogen for mouse and human cells and causes the development of Ig-producing cells. According to the publication, the effect was sequence specific because the antisense oligonucleotide to the P53 gene was inactive. The paper also indicates that a thioate backbone was essential as a diester did not work. It is noted in the discussion that the mice



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treated with the anti-*rev* oligonucleotide exhibited similar characteristics to humans infected with HIV, in that there was polyclonal B cell activation, decreased numbers of resting B cells, increased numbers of activated and fully differentiated B cells, and a decrease in response to mitogens.

Claim 20 was not obvious at the time of the invention in view of Branda et al., because the Branda reference does not teach that unmethylated CG oligonucleotides are useful for producing an antigen-specific immune response as claimed in claim 20. Branda teaches that an antisense oligonucleotide to the HIV *rev* gene causes B-cell activation. One of ordinary skill in the art would not believe that unmethylated CG oligonucleotides in combination with an antigen could produce an antigen-specific immune cell response based on the Branda disclosure.

New claim 32 recites a pharmaceutical composition of an *isolated* immunostimulatory nucleic acid of 8 to 100 nucleotides in length, having the following formula:

$$5' X_1CGX_2 3'$$

wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides, and an antigen. Claims 33-35 depend from claim 32.

New claim 36 recites a pharmaceutical composition of a *synthetic* immunostimulatory nucleic acid having the following formula:

$$5' X_1CGX_2 3'$$

wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides, and an antigen. Claims 37-39 depend from claim 36.

New claim 40 recites a pharmaceutical composition of an immunostimulatory nucleic acid of 8 to 40 nucleotides in length, having the following formula:

$$5' X_1CGX_2 3'$$

wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides, and an antigen. Claims 41-43 depend from claim 40.

Claims 32, 36, and 40 are not anticipated because none of the references listed above discloses an isolated oligonucleotide between 8 and 100 nucleotides in length, a synthetic oligonucleotide or an oligonucleotide between 8 and 40 nucleotides in length specifically in

combination with an antigen of interest. These claims are also not obvious in view of the references of record for each of the reasons set forth above.

Amended claims 12 and 14-19 recite various methods for treating a subject by administering an immunostimulatory nucleic acid, having the following formula:

$$5' X_1CGX_2 3'$$

wherein C and G are unmethylated, wherein $X_1$ and $X_2$ are nucleotides.

In particular, claim 12 recites a method for ameliorating an immune system deficiency in a subject by contacting and activating lymphocytes obtained from the subject with the immunostimulatory nucleic acid 5' $X_1CGX_2$ 3' *ex vivo*, in combination with an antigen, thereby producing activated lymphocytes; and readministering the activated lymphocytes to the subject. Claim 14 depends from claim 12.

None of the references of record disclose the *ex vivo* activation of lymphocytes using an immunostimulatory oligonucleotide and thus the claim is not anticipated. The method of amended claim 12 also would not have been obvious to one of ordinary skill in the art at the time of the invention because it would not have been obvious to administer an oligonucleotide in combination with antigen as described above.

Amended claim 15 recites a method for redirecting a subject's immune response from a Th2 to a Th1 by administering to the subject the immunostimulatory nucleic acid, wherein the nucleic acid has between only 8 and 100 nucleotides.

Amended claim 16 recites a method for ameliorating asthmatic symptoms in a subject, by administering to the subject the immunostimulatory nucleic acid 5' $X_1CGX_2$ 3'.

None of the references of record teach that the immunostimulatory oligonucleotides can redirect an immune response or ameliorate asthma and thus claims 15 and 16 are not anticipated. These methods would not have been obvious to one of ordinary sill in the art at the time of the invention because it would not have been obvious to administer an oligonucleotide to produce these effects.

Amended claim 17 recites a method for desensitizing a subject against the occurrence of an allergic reaction in response to contact with a particular allergen by administering to the subject an effective amount of the immunostimulatory nucleic acid' $X_1CGX_2$ 3' and an effective amount of the allergen.

Amended claim 18 recites a method of vaccination in a subject by administering to the subject a vaccine antigen or a nucleic acid encoding the vaccine antigen and the immunostimulatory nucleic acid.

As discussed above none of the references of record disclose the use of an immunostimulatory oligonucleotide and an antigen to produce an antigen-specific immune response. Thus claims 17 and 18 are not anticipated and are not obvious.

Amended claim 19 recites a method for treating leukemia in a subject by administering to the subject the immunostimulatory nucleic acid, prior to or in conjunction with a chemotherapy, so that the subject's leukemia cells are more sensitive to the chemotherapy. The use of an immunostimulatory oligonucleotide for rendering a leukemic cell more sensitive to chemotherapy is not disclosed or obvious in view of the prior art.

In summary, for the reasons set forth herein, Applicants maintain that claims 12, 14-19, and 20-47 clearly and patentably define the invention. Applicants respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

It is believed that the claims are now in condition for allowance. A favorable action is earnestly solicited. If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (617) 720-3500, x259.

Respectfully submitted,

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